EMBO Molecular Medicine

TECRL, a new life-threatening inherited arrhythmia gene associated with overlapping clinical features of both LQTS and CPVT

Harsha D Devalla, Roselle Gélinas, Elhadi H Aburawi, Abdelaziz Beqqali, Philippe Goyette, Christian Freund, Marie-A Chaix, Rafik Tadros, Hui Jiang, Antony Le Béchec, Jantine Monshouwer-Kloots, Tom Zwetsloot, Georgios Kosmidis, Frédéric Latour, Azadeh Alikashani, Maaike Hoekstra, Jurg Schlaepfer, Christine L Mummery, Brian Stevenson, Zoltan Kutalik, Antoine AF de Vries, Léna Rivard, Arthur AM Wilde, Mario Talajic, Arie O Verkerk, Lihadh Al-Gazali, John D Rioux, Zahurul A Bhuiyan, Robert Passier

Corresponding author: *Harsha Devalla & Robert Passier, Leiden University Medical Center*
Zahurul Bhuiyan, Universitaire Vaudois (CHUV), Lausanne
Jean Rioux, Montreal Heart Institute

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

Editor: Roberto Buccione

1st Editorial Decision 13 September 2015

Thank you for the submission of your manuscript to EMBO Molecular Medicine.

In this case we experienced unusual difficulties in securing three willing and appropriate reviewers, also due to the overlap with the vacation period. As a further delay cannot be justified I have decided to proceed based on the two available consistent evaluations.

Both Reviewers are quite positive on your manuscript although they raise some issues that require your action. I will not dwell into much detail as their comments are detailed. I would like, however, to highlight a few main points.

Reviewer 1, as you will see, lists a few of issues including that s/he suggests undertaking further experimentation to better define the role of TECRL, for instance by analysing the phenotype of "control" iPS cells with knock down of TECRL. We agree that to address this and the other points would significantly enhance the significance of the study.

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Reviewer 2 also suggests a number of actions to improve the manuscript. These include better statistical analysis (to this effect please see below on our checklist), streamlining the clinical details on the patients and others. Regarding the latter point, we agree and perhaps you might want to move some information to the supplementary information section. Reviewer 2, however, would also like you to experimentally address why you observed no noradrenaline-triggered delayed after depolarizations and whether this was due to the presence of the calcium indicator.

In conclusion, while publication of the paper cannot be considered at this stage, we would be pleased to consider a substantially revised submission, with the understanding that the Reviewers' concerns must be addressed with additional experimental data where appropriate and that acceptance of the manuscript will entail a second round of review.

Please note that it is EMBO Molecular Medicine policy to allow a single round of revision only and that, therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript.

As you might know, EMBO Molecular Medicine has a "scooping protection" policy, whereby similar findings that are published by others during review or revision are not a criterion for rejection. However, I do ask you to get in touch with us after three months if you have not completed your revision, to update us on the status. Please also contact us as soon as possible if similar work is published elsewhere.

Please note that EMBO Molecular Medicine now requires a complete author checklist (http://embomolmed.embopress.org/authorguide#editorial3) to be submitted with all revised manuscripts. Provision of the author checklist is mandatory at revision stage; The checklist is designed to enhance and standardize reporting of key information in research papers and to support reanalysis and repetition of experiments by the community. The list covers key information for figure panels and captions and focuses on statistics, the reporting of reagents, animal models and human subject-derived data, as well as guidance to optimise data accessibility.

I also suggest that you carefully adhere to our guidelines for publication in your next version, including presentation of statistical analyses (see also below) and our new requirements for supplemental data (Expanded View; http://embomolmed.embopress.org/authorguide#expandedview) to speed up the pre-acceptance process.

I look forward to seeing a revised form of your manuscript as soon as possible.

***** Reviewer's comments *****

Referee #1 (Remarks):

Devalla and colleagues generated iPSC from patients affected by a clinical CPVT syndrome to phenocopy this disease in the dish. Mutations of RyR2 (observed in CPVT1) and Casq2 (observed in CPVT2) were not identified. Whole genome sequencing revealed a deletion of exon 3 in the Tecrl gene. Association of Tecrl with the ER and strong expression in the developing mouse heart were demonstrated. Diastolic calcium overload potentially as a consequence of delayed cytosolic calcium clearance was observed and suggested to be the underlying cause of DAD-like electrophysiological events. Noradrenaline enhanced DADs as anticipated for cardiomyocytes from patients with CPVT.

Major concerns:

1) Additional experiments are needed to underpin the mechanistic role of Tecrl in the observed phenotype. This would also strengthen the rationale for including Tecrl in genetic panel for CPVT screens. Would a knock-down of Tecrl in a control ESC or iPSC line lead to a similar phenotype? Alternatively, would overexpression of exon 3 deleted Tecrl induce a similar phenotype?

2) Data on Tecrl transcripton and/or protein expression would be helpful to understand whether the

exon 3 deleted transcript/protein is present, absent, or compensated in cardiomyocytes by the non-affected allele in the TecrlHet cardiomyocytes.

3) Data on RyR2, Casq2, SERCA, PLB, NCX, LTCC protein abundance and phosphorylation should be provided to understand whether canonical calcium handling proteins are affected.

Minor concerns:

1) It should be noted in the discussion that the AP duration, independent of the condition, remains relatively short for a ventricular myocyte (APD90: <200 ms). This does not limit the value of the study, but in my view provides further arguments of the use of iPSC in disease modelling despite an apparently immature electrophysiological phenotype.

Referee #2 (Comments on Novelty/Model System):

This is a very thorough study and of significant interest. The high ranking for technical quality is justified for all but one minor omission in the calcium handling studies (se comments to authors. In terms of medical impact - I have ranked it medium rather than high, simply because this is likely to be extremely rare - so whilst it has significant scientific interest I doubt that very many people will come across this condition.

Referee #2 (Remarks):

In this study, Devalla and colleagues have elucidated the genetic basis of a rare but highly malignant form of CPVT. They have identified a new gene involved in inherited arrhythmia syndromes, TECRL, and largely determined the underlying molecular basis of the pathology in this family. The approach they have taken is very logical and straight-forward, but that should not underestimate the magnitude of what they have achieved in terms of drilling down into the underlying mechanism. I have no major concerns with the study but a few points that should be clarified.

1. In the abstract I would include a comment about the slower rise time of the calcium transients and the lower SR Calcium stores as evidenced by the decreased magnitude of caffeine-induced transients.
2. p6. I do not think that it is necessary to add in so much of the clinical data. Of most relevance to this study are the electrical phenotypes - I do not think the readers need to know all the agonising details of the patients' stays in ICU.
3. p14. The authors comment that a raised diastolic [Ca2+]i would predispose to DADs. However, a decreased NCX activity could reduce DADs - the authors should add a comment along these lines - and modify their conclusions in the discussion along the lines of despite the reduced NCX, the increase in diastolic calcium was sufficient to still result in DADs.
4. For me, the only slight weakness in the study was the lack of data for calcium transients in the presence of NA. It is possible that the extra buffering of [Ca2+]i in the presence of indo-I might abrogate any NA-triggered DADs. If the authors have tried these experiments and did not observe DADs - then they should say so and provide a possible explanation (such as buffering effects). If they have not tried them then I would strongly encourage them to do these experiments as they will be very interesting, particularly if they parallel the findings with the AP recordings.
5. p28. Statistics. It is well known that there is considerable variability in AP and calcium transient characteristics in hIPS CMs from one differentiation to the next and even from one plating to the next of the same cell lines. It is therefore very important that the authors have repeated measurements on cells derived from at least three separate differentiations. For completeness, can the authors include in the relevant figure legends details of how many cells from how many independent platings are included. For example in Figure 4, rather than reporting n=15-18 it would be better to sate n=4-6 from 3 different platings/differentiations with total of n=15-18 (or whatever the specific numbers are).
6. Figure 8: If y axis is percentage then I presume the valuers on these should be 0, 50, 100 and 150 (not 0, 0,5, 1.0, 1.5).

1st Revision - authors' response 11 January 2016

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Major concerns:

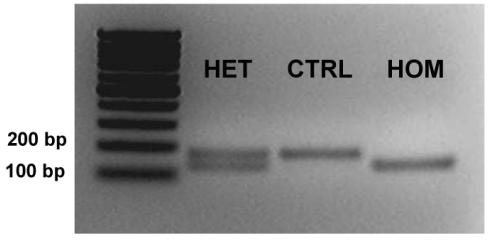
1) Additional experiments are needed to underpin the mechanistic role of Tecrl in the observed phenotype. This would also strengthen the rationale for including Tecrl in genetic panel for CPVT screens. Would a knock-down of Tecrl in a control ESC or iPSC line lead to a similar phenotype? Alternatively, would overexpression of exon 3 deleted Tecrl induce a similar phenotype?

We thank the reviewer for this question. As suggested, we performed shRNA-mediated knockdown of *TECRL* in control hESC-CMs, which resulted in a significant prolongation of action potential duration (APD) at 20%, 50% and 90% of repolarization (APD_{20}, APD_{50} and APD_{90}) compared with CMs treated with a scrambled shRNA (Fig. S5). This is in agreement with our findings in patient-derived hiPSC-CMs with a *TECRLc.331+1G>A* mutation, which also displayed significantly prolonged action potentials at 20% repolarization (APD_{20}) and there was a clear trend for an increase in APD at 50% (APD_{50}) and 90% (APD_{90}) repolarization compared to CTRL-hiPSC-CMs (Fig. 6C). Furthermore, hESC-CMs with *TECRL* knockdown showed decreased RYR2 (by 56%) and CASQ2 (by 18%) protein levels (Fig. S5E) similar to hiPSC-CMs with *TECRLc.331+1G>A* mutation (Fig. S4). These results suggest a link between *TECRL* and calcium homeostasis in CMs. Additionally, we also tried to evaluate the susceptibility of hESC-CMs with *TECRL* knockdown, to triggered activity. As done with hiPSC-CMs, we used a fast pacing episode (3-Hz; 10-seconds), followed by a 10-second pause in the absence or presence of 10 nM Noradrenaline (NA). However, due to extremely long APs of TECRL knockdown CMs, most cells were not able to follow a fast-pacing protocol and therefore, we could not provide this data in the manuscript.

We have included the results from *TECRL* knockdown experiments in the revised version of the manuscript (Results: Page 14-16 of main manuscript; Figure: S5 of supplementary information).

2) Data on Tecrl transcripton and/or protein expression would be helpful to understand whether the exon 3 deleted transcript/protein is present, absent, or compensated in CMs by the non-affected allele in the TecrlHet cardiomyocytes.

In $TECRL_{Het}$-hiPSC-CMs, we observed the presence of both *TECRL* transcripts by RT-PCR with primers designed to target exons 2-4 of *TECRL*: a longer 171bp product containing exon3 and a shorter 126 bp product lacking exon3 (Fig. 3G). So, it appears that the wild-type transcript does not compensate for the mutated transcript in $TECRL_{Het}$-hiPSC-CMs.



We also attempted to detect TECRL protein on western blot and immunostainings using a TECRL specific antibody. Unfortunately, both commercially available and custom-made antibodies were unsuccessful in detecting TECRL protein.

3) Data on RyR2, Casq2, SERCA, PLB, NCX, LTCC protein abundance and phosphorylation should be provided to understand whether canonical calcium handling proteins are affected.

We thank the reviewer for this question and these experiments have been very informative. By western blot, we assessed protein expression of several calcium handling proteins in hiPSC-CMs as well as in TECRL-knockdown CMs. RYR2 (by 52%) and CASQ2 (by 85%) were found to be consistently downregulated in $TECRL_{Hom}$-hiPSC-CMs and representative blots are shown in Fig. S4. However, we did not observe any differences in the protein levels of SERCA, PLB, NCX or CAv1.2 between CTRL and $TECRL_{Hom}$-hiPSC-CMs. Similarly, we also observed a reduction in the protein expression of RYR2 (by 56%) and CASQ2 (by 18%) in *TECRL*-knockdown CMs (Fig. S5E). These results suggest that the *c.331+1G>A* mutation or a deficiency in *TECRL* influences the expression of RYR2 and CASQ2, which have both been implicated in CPVT.

However, WB to detect phosphorylated RYR2 or phosphorylated PLB were not successful likely due to relatively low expression of these proteins in iPSC- and hESC-derived CMs. Another explanation of the lack of phospho-specific signal could be that the phosphatase inhibition by NaF and Na_3VO_4 was not sufficient. To our knowledge, there has not been any published data on detection of phosphorylated calcium handling proteins in hPSC-derived CMs.

We have included the results from WB of canonical calcium handling proteins in the revised version of the manuscript (Results: Page-12; 15-16 of main manuscript; Figure: S4; S5E of supplementary information).

Minor concerns:

1) It should be noted in the discussion that the AP duration, independent of the condition, remains relatively short for a ventricular myocyte (APD90: <200 ms). This does not limit the value of the study, but in my view provides further arguments of the use of iPSC in disease modelling despite an apparently immature electrophysiological phenotype.

The reviewer is right that the APD in PSC-derived CMs is shorter than in freshly isolated adult ventricular CMs. We have mentioned this point in the discussion (Page-22 of main manuscript) and that this does not preclude their use in revealing disease phenotypes

Referee #2 (Comments on Novelty/Model System):

This is a very thorough study and of significant interest. The high ranking for technical quality is justified for all but one minor omission in the calcium handling studies (se comments to authors. In terms of medical impact - I have ranked it medium rather than high, simply because this is likely to be extremely rare - so whilst it has significant scientific interest I doubt that very many people will come across this condition.

Referee #2 (Remarks):

In this study, Devalla and colleagues have elucidated the genetic basis of a rare but highly malignant form of CPVT. They have identified a new gene involved in inherited arrhythmia syndromes, TECRL, and largely determined the underlying molecular basis of the pathology in this family. The approach they have taken is very logical and straight-forward, but that should not underestimate the magnitude of what they have achieved in terms of drilling down into the underlying mechanism. I have no major concerns with the study but a few points that should be clarified.

1. In the abstract I would include a comment about the slower rise time of the calcium

transients and the lower SR Calcium stores as evidenced by the decreased magnitude of caffeine-induced transients.

We thank the reviewer for this suggestion and have now included this information in the abstract (Page-3 of the main manuscript).

2. p6. I do not think that it is necessary to add in so much of the clinical data. Of most relevance to this study are the electrical phenotypes - I do not think the readers need to know all the agonising details of the patients' stays in ICU.

As suggested by the reviewer and the editors, we have moved most of the clinical data to the supplementary section. Brief description of subject IV:13 (also investigated by hiPSCs in this study) and a table summarizing the other patients in the family have been left in the main manuscript.

3. p14. The authors comment that a raised diastolic [Ca2+]i would predispose to DADs. However, a decreased NCX activity could reduce DADs - the authors should add a comment along these lines - and modify their conclusions in the discussion along the lines of despite the reduced NCX, the increase in diastolic calcium was sufficient to still result in DADs.

The reviewer raises a valid point and we have now included this in the discussion (Page-20 of main manuscript).

4. For me, the only slight weakness in the study was the lack of data for calcium transients in the presence of NA. It is possible that the extra buffering of [Ca2+]i in the presence of indo-I might abrogate any NA-triggered DADs. If the authors have tried these experiments and did not observe DADs - then they should say so and provide a possible explanation (such as buffering effects). If they have not tried them then I would strongly encourage them to do these experiments as they will be very interesting, particularly if they parallel the findings with the AP recordings.

The reviewer raises an important point, which we also recognized during our study. We tried extensively to measure spontaneous Ca^{2+}-release in hiPSC-CMs in response to NA, with a protocol similar to our patch clamp experiments. Our intention was therefore, to use a fast pacing episode (3-Hz; 10-seconds), followed by a 10-second pause in the absence or presence of 10 nM Noradrenaline (NA). Unfortunately we were not able to pace hiPSC-CMs with field stimulation at 3 Hz and had to discontinued this approach. It is a general observation that it is more difficult to pace CMs at fast frequencies with field stimulation. The reviewer is also right that extra buffering of $[Ca^{2+}]$i may occur in the presence of Indo-I. We also observed that hiPSC-CMs become quiescent after Indo-1 loading, as also mentioned in the methods section (page-6 of supplementary information) and this additionally might have hampered the measurements.

5. p28. Statistics. It is well known that there is considerable variability in AP and calcium transient characteristics in hIPS CMs from one differentiation to the next and even from one plating to the next of the same cell lines. It is therefore very important that the authors have repeated measurements on cells derived from at least three separate differentiations. For completeness, can the authors include in the relevant figure legends details of how many cells from how many independent platings are included. For example in Figure 4, rather than reporting n=15-18 it would be better to sate n=4-6 from 3 different platings/differentiations with total of n=15-18 (or whatever the specific numbers are).

As the reviewer rightly points out, variation in electrical and functional parameters can be noted between differentiation to differentiation from hiPSC-lines. Data presented in this study for all of the experiments (electrophysiology, calcium measurements and WBs included in the revised manuscript) were acquired from at least three independent differentiations. For clarity, this is also mentioned in the subsection ‘statistics’ of the methods in the main manuscript (Page-25 of main manuscript). As suggested by the reviewer, figure legends for individual figures also now contain additional information about the number of cells obtained per experiment.

6. Figure 8: If y axis is percentage then I presume the values on these should be 0, 50, 100 and 150 (not 0, 0,5, 1.0, 1.5).

We thank the reviewer for bringing this to our notice. The values on y-axis in Fig.8B and 8D are indeed a percentage and have now been corrected

2nd Editorial Decision 26 January 2016

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine. We have now received the enclosed reports from the referees that were asked to re-assess it. As you will see the reviewers are now globally supportive and I am pleased to inform you that we will be able to accept your manuscript pending the following final amendments:

1) Please comply with Reviewer 2's final request for a minor clarification

2) The manuscript is still missing "The paper explained" section". EMBO Molecular Medicine articles are accompanied by a summary of the articles to emphasize the major findings in the paper and their medical implications for the non-specialist reader. Please provide a draft summary of your article highlighting the medical issue you are addressing, the results obtained and their clinical impact. Please refer to any of our published papers as a reference (embomolmed.org). This may be edited to ensure that readers understand the significance and context of the research. Please refer to any of our published articles for an example.

3) As per our Author Guidelines, the description of all reported data that includes statistical testing must state the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments underlying each data point (not replicate measures of one sample), and the actual P value for each test (not merely 'significant' or 'P < 0.05').

4) We are now encouraging the publication of source data, particularly for electrophoretic gels and blots, with the aim of making primary data more accessible and transparent to the reader. Would you be willing to provide a PDF file per figure that contains the original, uncropped and unprocessed scans of all or at least the key gels used in the manuscript? The PDF files should be labeled with the appropriate figure/panel number, and should have molecular weight markers; further annotation may be useful but is not essential. The PDF files will be published online with the article as supplementary "Source Data" files. If you have any questions regarding this just contact me.

5) Every published paper includes a 'Synopsis' to further enhance discoverability. Synopses are displayed on the journal webpage and are freely accessible to all readers. They include a short standfirst as well as 2-5 one sentence bullet points that summarise the paper. Please provide the synopsis including the short list of bullet points that summarise the key NEW findings. The bullet points should be designed to be complementary to the abstract - i.e. not repeat the same text. We encourage inclusion of key acronyms and quantitative information. Please use the passive voice. Please attach this information in a separate file or send them by email, we will incorporate it accordingly. You are also welcome to suggest a striking image or visual abstract to illustrate your article. If you do please provide a jpeg file 550 px-wide x 400-px high.

6) As per our guidelines for Authors, the figure call-outs within the article and inside "suppl. information" need to be adjusted to Appendix Figure S1, Appendix Figure S2, etc ... and the supplementary information file must be renamed "Appendix".

7) Please note that we now mandate that all corresponding authors list an ORCID digital identifier. You may do so though our web platform upon submission and the procedure takes <90 seconds to complete. We encourage all authors to supply an ORCID identifier, which will be linked to their name for unambiguous name identification.

Please submit your revised manuscript within two weeks. I look forward to seeing a revised form of your manuscript as soon as possible.

***** Reviewer's comments *****

Referee #1 (Remarks):

The authors have addressed my critiques.

Referee #2 (Remarks):

The authors have addressed all of the concerns I raised. The one very minor point I would like to address - is that they indicate in the main manuscript that they did try to measure spontaneous release events in response to NA (to complement the electrical measurements) but were not able to do so due to the technical limitations of using field stimulation (rather than direct pacing) (as indicated in their response to my previous point 4). This could either be mentioned at the end of the relevant results section (p16) or in the discussion.

2nd Revision - authors' response 27 July 2016

On behalf of all the authors, we thank you for the feedback on earlier versions of our manuscript titled "A splice site mutation in *TECRL* is associated with inherited lethal arrhythmias in humans". Based on your comments, we had revised the article in January this year that was then accepted.

However, during the evaluation of the previous version, we learnt that the group of John Rioux from the University of Montreal in Canada also identified patients who carried a rare missense homozygous mutation in *TECRL* and a clinical phenotype of LQTS but also adrenergic-induced arrhythmias. In the Sudanese family we had presented in our study earlier, we had observed adrenergic-induced arrhythmias in patients with a splice-site mutation in *TECRL* who were clinically diagnosed with CPVT but QTc prolongation was also noted in some patients.

This confirmed our hypothesis that indeed *TECRL* is an import gene associated with stress-induced arrhythmias (with overlapping features of both LQTS and CPVT, also evident in patient-derived hiPSC-cardiomyocytes and knockdown experiments). We felt that adding clinical data from unrelated patients would maximize the clinical impact with the message that genetic testing for mutations in *TECRL* should be implemented in patients negative for mutations in classic LQTS and CPVT genes. Also, we wanted to present the story as best as possible with all of the information known to us at this point and hence took the decision of adding the clinical data from Canada to our existing data.

We apologize for the delay in resubmission of the combined manuscript, which was due to collecting additional patient data and organizing the manuscript but we are now confident that the message of the study is stronger with the inclusion of multiple unrelated patients. Findings presented in our manuscript add to the spectrum of LQTS and CPVT associated genes that can be implemented in diagnostic screenings. Our work also reiterates the value of hiPSC derivatives to study human disorders, rare diseases in particular. The revised manuscript is now titled "*TECRL*, a new lifethreatening inherited arrhythmia gene associated with overlapping clinical features of both LQTS and CPVT ".

In this new version, Figure-1 has been modified to present clinical features of three different patients. In Figure-2, we have replaced the panel showing expression of *TECRL* in adult mouse tissues with expression of *TECRL* in human tissues. The other figures and results remain unchanged. Text in parts of the manuscript has been adapted to fit the new information in the revised version.

We thank you for your time and look forward to your input.

3rd Editorial Decision 18 August 2016

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine. We have now received the enclosed report from reviewer 1 who was asked to re-assess it also on behalf of reviewer 2, who was not available.

As you will see the reviewer is satisfied with your amended and integrated manuscript (but please note his/her remark on figure labeling) and I am pleased to inform you that we will be able to accept your manuscript pending the following final amendments. In fact, although I had mentioned many of them in my previous decision letter, I note that they have not yet been dealt with:

1) Please update the author list in the manuscript submission interface when you upload your revised manuscript

2) The manuscript is still missing "The paper explained" section". EMBO Molecular Medicine articles are accompanied by a summary of the articles to emphasize the major findings in the paper and their medical implications for the non-specialist reader. Please provide a draft summary of your article highlighting the medical issue you are addressing, the results obtained and their clinical impact. Please refer to any of our published papers as a reference (embomolmed.org). This may be edited to ensure that readers understand the significance and context of the research. Please refer to any of our published articles for an example.

3) As per our Author Guidelines, the description of all reported data that includes statistical testing must state the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments underlying each data point (not replicate measures of one sample), and the ACTUAL P value for each test (not merely 'significant' or 'P < 0.05').

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5) Every published paper includes a 'Synopsis' to further enhance discoverability. Synopses are displayed on the journal webpage and are freely accessible to all readers. They include a short standfirst as well as 2-5 one sentence bullet points that summarise the paper. Please provide the synopsis including the short list of bullet points that summarise the key NEW findings. The bullet points should be designed to be complementary to the abstract - i.e. not repeat the same text. We encourage inclusion of key acronyms and quantitative information. Please use the passive voice. Please attach this information in a separate file or send them by email, we will incorporate it accordingly. You are also welcome to suggest a striking image or visual abstract to illustrate your article. If you do please provide a jpeg file 550 px-wide x 400-px high.

6) Please note that EMBO Molecular Medicine now requires a complete author checklist (http://embomolmed.embopress.org/authorguide#editorial3) to be submitted with all revised manuscripts. Provision of the author checklist is mandatory at revision stage; The checklist is designed to enhance and standardize reporting of key information in research papers and to support reanalysis and repetition of experiments by the community. The list covers key information for figure panels and captions and focuses on statistics, the reporting of reagents, animal models and human subject-derived data, as well as guidance to optimise data accessibility. The Author checklist will be published alongside the paper, in case of acceptance, within the transparent review process file.

I look forward to seeing a revised form of your manuscript as soon as possible, and in any case within two weeks, so that we can rapidly proceed with formal acceptance and production.

***** Reviewer's comments *****

Referee #1 (Remarks):

The additional clinical and experimental data from Montreal strongly supports the hypothesis that TECRL mutations are associated with ventricular arrhythmia. The authors must be congratulated on their efforts to further strengthen the translational relevance of their work.

On page 15 Fig 3H should be 3G.

EMBO MOLECULAR MEDICINE

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND

Corresponding Author Name: H.D.Devalla
Manuscript Number: EMM-2015-05719-V3

Reporting Checklist For Life Sciences Articles

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript (see link list at top right).

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars only for independent experiments and sample sizes where the application of statistical tests is warranted (error bars should not be shown for technical replicates)
- when n is small (n < 5), the individual data points from each experiment should be plotted alongside an error bar.
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation (see link list at top right).

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

USEFUL LINKS FOR COMPLETING THIS FORM

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http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

B- Statistics and general methods

Please fill out these boxes

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	We have performed extensive studies of this type in the past. The group size is the minimum number that is sufficient to reliably define a specific functional parameter taking into account the biological variability in electrophysiological properties.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	Not applicable
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Variability in electrophysiological and molecular parameters can be expected from differentiation to differentiation in hPSC-CMs. Therefore, samples were not excluded from analysis based on any pre-established criterion.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	Experimental intervention was not randomized.
For animal studies, include a statement about randomization even if no randomization was used.	Not Applicable
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	Patch-clamp/calcium/WB experiments on different groups were performed blinded
4.b. For animal studies, include a statement about blinding even if no blinding was done	Not Applicable
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	The 'SigmaStat software v3.5' was used for statistical analysis. The software test normality on data sets using the Kolmogorov-Smirnov test.This information is stated in the Methods section of the main manuscript on page-25.
Is there an estimate of variation within each group of data?	The 'SigmaStat software v3.5' was used for statistical analysis. The software performed a test for equal variance assumption on data sets using the Levene median test. This information is stated in the Methods section of the main manuscript on page-25.
Is the variance similar between the groups that are being statistically compared?	Not always. Groups were compared using 1-way ANOVA followed by pairwise comparison using the Student-Newman-Keuls test or, in cases of failed normality and/or equal variance test, Kruskal-Wallis test followed by Dunn's test.This information is stated in the Methods section of the mainmanuscript on page-25.

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	RYR2: clone C3-33,Thermo Scientific; CASQ2: clone E-12, Santa Cruz; SERCA2a: Badrilla; PLB: clone A1, Badrilla; NCX:clone C2C12, Thermo Scientific; Cav1.2: Alomone labs; ACTN2: clone EA-53, Sigma; GAPDH: clone 6C5, Fitzgerald. This information along with antibody dilutions used is stated in the 'Methods' on page-8 of 'Supplementary Information'
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	hiPSC lines reported in this study were reprogrammed from skin fibroblasts using non-integrating sendai viruses. Official identification of cell lines used in this study are LU0046iTECRL (heterozygous c.331+1G>A mutation in TECRL), LU0047iCTRL (control line) and LU0048iTECRL (homozygous c.331+1G>A mutation in TECRL). Cells in culture were regularly tested for mycoplasma.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	Not Applicable
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Not Applicable
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines' (see link list at top right). See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	Not Applicable

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	Skin biopsies were obtained from consenting patients or their guardians and the research protocol was approved by the Al-Ain Medical District Human Research Ethics Committee, College of Medicine, United Arab Emirates University. Methods section on page-21 of 'Main' manuscript contains this information.
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	Studies reported here conform to the principles outlined in the Declaration of Helsinki, were reviewed and approved by relevant ethics Committees. Human subjects gave written informed consent. This information is stated in the 'Methods' section on page-22 of 'Main' manuscript
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	Not Applicable
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	Not Applicable
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	Not Applicable
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines' (see link list at top right).	Not Applicable
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines' (see link list at top right).	Not Applicable

F- Data Accessibility

18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition' (see link list at top right). Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	Not Applicable
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	Not Applicable
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	Not Applicable
21. As far as possible, primary and referenced data should be formally cited in a Data Availability section: Examples: **Primary Data** Wetmore KM, Deutschbauer AM, Price MN, Arkin AP (2012). Comparison of gene expression and mutant fitness in Shewanella oneidensis MR-1. Gene Expression Omnibus GSE39462 **Referenced Data** Huang J, Brown AF, Lei M (2012). Crystal structure of the TRBD domain of TERT and the CR4/5 of TR. Protein Data Bank 4O26 AP-MS analysis of human histone deacetylase interactions in CEM-T cells (2013). PRIDE PXD000208	Not Applicable
22. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	Not Applicable

G- Dual use research of concern

23. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	'Dual use research of concern' does not apply to this study.